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L26: Entry 6 of 6 File: USPT Jun 4, 2002

DOCUMENT-IDENTIFIER: US 6399747 B1
TITLE: Shc-binding protein

Detailed Description Text (3):
The predicted amino acid sequence encoded by mPAL DNA contains 23 tyrosine residues, several of which are embedded in consensus binding motifs for SH2 domains. In addition, two highly acidic regions are encoded by the mPAL DNA. Comparison of both the nucleotide and protein sequences of mPAL with the GenBank databases revealed no significant homology between mPAL and any previously identified proteins. We have identified several related expressed sequence tags (ESTs) represent human and rat homologues of mPAL. In addition several short murine and human ESTs with approximately 50% sequence similarity to regions of mPAL were identified, suggesting that additional mPAL related genes exist.

Detailed Description Text (16):
The full length PAL polypeptide or fragment thereof can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook et al. Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and/or Ausubel et al., eds, Current Protocols in Molecular Biology, Green Publishers Inc. and Wiley and Sons, NY (1994). A gene or cDNA encoding the PAL protein or fragment thereof may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Alternatively, a gene encoding the PAL polypeptide or fragment may be prepared by chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al., Angew. Chem. Intl. Ed., 28:716-734 (1989). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphorate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the PAL polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length PAL polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, that encodes a methionine residue. This methionine may or may not be present on the mature form of the PAL polypeptide, depending on whether the polypeptide produced in the host cell is secreted from that cell.

Detailed Description Text (17):
In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants or analogs of naturally occurring PAL. Nucleic acid variants or analogs (wherein one or more nucleotides and/or amino acids are designed to differ from the wild-type or naturally occurring PAL) may be produced using site directed mutagenesis or PCR amplification where the primer(s) have the desired point mutations (see Sambrook et al., supra, and Ausubel et al., supra, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels et al., supra, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well. For example, in Wayne et al., EMBO J 2:1827-1829 (1983), the authors teach a method for deletion mutagenesis, that was used to generate mutants of the TyrTS gene. Huang et al., Cell 48:129-136 (1987), analyzed

the functional domains of Pseudomonas exotoxin using a deletion analysis of the gene expressed in E. coli. In 1986, Zumstein et al., J. Mol. Biol. 191: 333-340, described the analysis of structural and functional domains of E. coli DNA Topoisomerase I using insertion and deletion mutagenesis. In DeChiara et al., Methods in Enzymol. 119:403-415, the authors describe procedures for in vitro DNA mutagenesis of human leukocytes interferon. Other publications describing mutagenesis of cloned genes and subsequent testing of the polypeptide encoded thereby include Doyle et al., J. Cell Biol. 103:1193-1204 (1986), and others. Preferred nucleic acid variants or analogs are those containing nucleotide substitutions accounting for codon preference in the host cell that is to be used to produce PAL. Other preferred variants or analogs are those encoding conservative amino acid changes as described above (e.g., wherein the charge or polarity of the naturally occurring amino acid side chain is not altered substantially by substitution with a different amino acid), as compared to wild type, analogs of PAL polypeptide(s), and/or those designed to either generate a novel glycosylation and/or phosphorylation site(s) on PAL, or those designed to delete an existing glycosylation and/or phosphorylation site(s) on PAL.

Detailed Description Text (24):
Typically, the vectors used in any of the host cells will comprise a promoter operatively linked usually to the 5' end of a DNA molecule to be expressed. Vectors also typically comprise other regulatory elements as well such as an enhancer(s), an origin of replication element, a transcriptional termination element, a complete intron sequence containing a donor and acceptor splice site, a signal peptide sequence, a ribosome binding site element, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these elements is discussed below. Optionally, the vector may contain a "tag" sequence, i.e., an oligonucleotide sequence located at the 5' or 3' end of the PAL coding sequence that encodes poly-Histidine (such as hexahis), or another small sequences which may be immunogenic or which may have other biological properties such as the ability to prolong the half-life of the polypeptide or to target the polypeptide to cells, organelles or ligands. This tag will be expressed along with the protein, and can serve as an affinity tag for purification of the PAL polypeptide from the host cell. Optionally, the tag can subsequently be removed from the purified PAL polypeptide by various means such as using a selected peptidase for example.

Detailed Description Text (42):
Selection of the host cell will depend in part on whether the PAL protein is to be glycosylated or phosphorylated (in which case eukaryotic host cells are preferred), and the manner in which the host cell is able to "fold" the protein into tertiary structure (e.g., proper orientation of disulfide bridges, etc.) such that biologically active protein is produced. However, where the host cell does not synthesize properly folded biologically active PAL, the PAL may be "folded" after synthesis using appropriate chemical conditions as discussed below. It is also well known in the art that the host cell in which a PAL encoding DNA molecule is expressed will affect the glycosylation pattern of the expressed protein with the result being that the protein expressed in a host cell other than that in which it is normally expressed will have a different (e.g. non-native), glycosylation pattern. However, such polypeptides may still maintain full or partial biological activity.

Detailed Description Text (52):
Purification of PAL polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as hexahistidine (PAL/hexaHis), or other small peptide at either its carboxyl or amino terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (i.e., a monoclonal antibody specifically recognizing PAL). For example, polyHistidine binds with great affinity and

specificity to nickel, thus an affinity column of nickel (such as the Qiagen nickel columns) can be used for purification of PAL/polyHis. (See for example, Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York (1993)).

Detailed Description Text (53):
Where the PAL polypeptide has no tag, and where there are no anti-PAL antibodies available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of any of the foregoing techniques may be combined to achieve increased purity. Preferred methods for purification include polyHistidine tagging and ion exchange chromatography in combination with preparative isoelectric focusing.

Detailed Description Text (77):
For determining the effect on PAL/Shc protein binding or PAL production, one can remove proteins from the cell or sample and use methods similar to those above that tag or isolate PAL/Shc protein complexes or PAL. For example, one could use immunoaffinity purification technique, which may or may not include the use of fusion constructs for the PAL and Shc.

Other Reference Publication (78):
Zumstein, L. et al., "Probing the Structural Domains and Function in Vivo of Escherichia coli DNA Topoisomerase I by Mutagenesis," J. Mol. Biol., 191:333-340 (1986).

Other Reference Publication (82):
Adams, M.D. et al., "Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library," Nature Genetics, 4:373-380 (Aug., 1993).